

**KARAKTERISASI MOLEKULAR DAN SENYAWA BIOAKTIF
STREPTOMYCES DARI RHIZOSFER RUMPUT TEKI
(*Cyperus rotundus* L.) DI DATARAN TINGGI CEMORO
SEWU, MAGETAN, JAWA TIMUR**

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INTISARI

Streptomyces dikenal sebagai mikroorganisme penghasil senyawa bioaktif terbesar terutama antibiotik. Tujuan penelitian untuk mengisolasi dan mengkarakterisasi secara molekular melalui *Whole Genome Sequencing* (WGS) *Streptomyces* spesies baru penghasil antibiotik serta mengkarakterisasi senyawa bioaktif yang dihasilkannya. Sampel tanah diambil dari rhizosfer Rumput Teki (*Cyperus rotundus* L.) di Dataran Tinggi Cemoro Sewu, Magetan, Jawa Timur dengan ketinggian 1920 mdpl. Penelitian dilakukan sebagai berikut: (1) isolasi, purifikasi dan uji aktivitas antibiotik isolat *Streptomyces*, (2) karakterisasi isolat *Streptomyces* penghasil antibiotik, serta (3) karakterisasi senyawa bioaktif yang dihasilkan oleh isolat *Streptomyces* unggul. Kerapatan populasi *Streptomyces* yang tumbuh pada medium SCA sebesar $3,9 \times 10^7$ CFU/g, sedangkan pada medium RHA sebesar $3,5 \times 10^7$ CFU/g. Di antara 45 isolat murni hasil purifikasi, sebanyak 28 isolat (62,22%) terbukti dapat menghambat pertumbuhan mikroorganisme uji. Berdasarkan potensi penghambatannya sebanyak enam isolat ditetapkan sebagai isolat *Streptomyces* terpilih. Hasil analisis sekuen gen 16S rRNA menunjukkan satu isolat yang paling potensial (*Streptomyces* sp. CRB46) berkerabat sangat dekat dengan *S. rochei* NRRL B-2410 (kemiripan 100%). Meskipun demikian analisis sekuen hasil WGS menunjukkan bahwa tingkat kemiripan antara *Streptomyces* sp. CRB46 dengan *S. rochei* NRRL B-2410 hanya 95,31%. Oleh karena itu kedua *Streptomyces* dapat dianggap sebagai spesies yang berbeda. Untuk mendukung perbedaan kedua spesies *Streptomyces* maka dilakukan analisis hasil WGS dengan tiga *software* berbeda. Hasil RAST menunjukkan bahwa *Streptomyces* sp. CRB46 memiliki ukuran genom 8.197.669 bp, jumlah subsistem 347, jumlah sekuen pengkode 7.714 (0,094%) dan jumlah gen hipotetik 2.559 (33,17%). Sedangkan pada *S. rochei* NRRL B-2410 jumlah masing-masing sebesar 7.682.624 bp, 344 subsistem, 7.110 (0,093%) dan 2.204 (31,00%). Hasil analisis AntiSMASH menunjukkan bahwa *Streptomyces* sp. CRB46 memiliki jumlah kelompok gen penghasil senyawa bioaktif 53 kelompok, total jenis senyawa bioaktif 40 jenis, total gen PKS 20 gen, jumlah kelompok gen atau golongan senyawa bioaktif dengan kemiripan 100% sebanyak 12 kelompok, jumlah kelompok gen atau golongan senyawa bioaktif dengan kemiripan 0% sebanyak sembilan kelompok, dan

senyawa bioaktif khusus nukleosida (malasidin). Sementara itu pada *S. rochei* NRRL B-2410 masing-masing sebesar 41 kelompok, 34 jenis, 15 gen, tujuh kelompok, tiga kelompok, dan lassopeptida (anantin). Hasil analisis BASys menunjukkan bahwa ukuran *Streptomyces* sp.CRB46 sekitar 8,2 Mbp, sedangkan *S. rochei* NRRL B-2410 sebesar 7,68 Mbp. Selain itu posisi gen PKS dari kedua *Streptomyces* berbeda. Hasil uji bioautografi menunjukkan ekstrak EAS (ekstraselular) dengan Rf 1,00, memiliki daya hambat terhadap mikroorganisme uji. Berdasarkan hasil LCMS diketahui EAS memiliki 20 campuran senyawa bioaktif. Tiga senyawa utama memiliki berat molekul sebesar 163,02 g/mol, 149,02 dan 301,14 g/mol serta 102,02 dan 111,02 g/mol. Hasil FTIR menunjukkan EAS mengandung gugus : amina, alkana, senyawa aromatik, imina/okima, asam karboksilat, fenol, amina aromatik, alkil, aril, eter, alkohol sekunder, anhidrid, 1,3-disubstitusi/1,2,4-trisubstitusi dan 1,4 disubstitusi/ 1,2,3,4 tetrasubstitusi. Sedangkan hasil AntiSMASH menunjukkan *Streptomyces* sp. CRB46 memiliki enam jenis senyawa yang strukturnya mirip 100% dengan senyawa yang telah ada, yaitu : isorenieraten, ektoin, melanin, albaflavenon, geosmin dan 7-prenilisatin. Berdasarkan hasil penelitian ini disimpulkan bahwa *Streptomyces* sp. CRB46 merupakan spesies *Streptomyces* baru, dengan potensi sebagai antibakteri dan anticandida serta diusulkan namanya sebagai *Streptomyces cemorosewuensis* sp. Nov.

Kata Kunci : *Streptomyces*, Rhizosfer, Dataran Tinggi, Senyawa Bioaktif, *Whole Genome Sequencing*

**MOLECULAR CHARACTERISATION OF *STREPTOMYCES* ISOLATED
FROM THE RHIZOSPHERE OF *Cyperus rotundus* L. OF CEMORO SEWU
HIGHLAND, MAGETAN, EASTERN JAVA AND ITS
BIOACTIVE COMPOUNDS**

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ABSTRACT

Streptomyces is a member of Actinomycetes widely known for its ability to produce secondary metabolites as part of bioactive compounds, especially antibiotics. The goal of the research were to isolate and characterise novel antibiotic-producing *Streptomyces* from rhizosphere of *Cyperus rotundus* L. by employing Whole Genome Sequencing (WGS) approach and elucidating the secondary metabolites synthesised. Soil samples were taken from the rhizosphere of *Cyperus rotundus* L. in the Cemoro Sewu highland in Magetan, East Java with an altitude of 1920 meters above sea level. The study consists of three main steps: (1) Isolation, purification and antibiotic activity tests of *Streptomyces* isolates against test microorganisms, (2) Phenetic and phylogenetic characterisation of *Streptomyces* isolates, (3) Characterisation of secondary metabolite bioactive compounds, produced by the selected *Streptomyces* isolates. The results of the research showed that the density of *Streptomyces* grew on SCA and RHA media were 3.9×10^7 CFU/g, and 3.5×10^7 CFU/g, respectively. Among 45 isolates obtained, 28 isolates (62.22%) were found inhibited the growth of test microorganisms. A total of six isolates were subsequently selected for further analysis. Results of phylogenetic characterisation suggested that CRB46 were sister clades of *Streptomyces rochei* strain NRRL B 2410 with 100% similarity levels. Even so The WGS sequences showed that the degree of similarity between *Streptomyces* sp. CRB46 with *S. rochei* NRRL B 2410 was 95.31%. The WGS results thus confirmed that *Streptomyces* sp. CRB46 is a different from *S. rochei* NRRL B 2410. Further confirmation that was obtained by RAST, AntiSMASH and BASys analysis. Genome length and number of subsystems on *Streptomyces* sp. CRB46 were 8,197,669 bp and 347 subsystems respectively. On the other hand, *S. rochei* NRRL B 2410 showed genome length and number of subsystems of 7,682,624 bp and 344 subsystems, respectively. the number of coding sequences and the number of hypothetical genes in *Streptomyces* sp. CRB46 were 7,714 (0.094%) and 2,559 (33.17%), while 7,110 (0.093%) coding sequences and 2,204 (31.00%) of hypothetical genes were present in the genome of *S. rochei* NRRL B 2410. AntiSMASH analysis results also showed differences between *Streptomyces* sp. CRB46 and *S. rochei* NRRL B 2410. The number of gene clusters, types of secondary metabolite bioactive compounds, and the number

of PKS genes in *Streptomyces* sp. CRB46 were 53 clusters, 40 species and 20 genes, respectively. Meanwhile in *S. rochei* NRRL B 2410 there were 41 clusters, 34 species and 15 genes. *Streptomyces* sp. CRB46 had eight compounds which showed 100% similarity with the known compounds, while *S. rochei* NRRL B 2410 only had seven of such compounds. One type of compound that is only found in *Streptomyces* sp. CRB46 is micromonolactam.

It was also found that nine gene clusters have never been found in other *Streptomyces* (0% similarity level). In *S. rochei* NRRL B 2410, however, there are three gene clusters. BASys analysis results show that the length of the *Streptomyces* sp. CRB46 genome is around 8.2 Mbp, while that of *S. rochei* NRRL B 2410 is around 7.68 Mbp. The bioautographic results showed that TLC chromatogram spot on EAS had an R_f of 1.00. LCMS results showed that EAS consisted of 20 mixtures of organic compounds with three main compounds with molecular weight 163.02 g/mol, 149.02, 301.14, 102.02 and 111.02 g/mol. FTIR results showed that EAS had an IR spectrum of 16 peaks. It is predicted that bioactive compounds contained in EAS include: amines, alkanes, aromatic compounds, imines/okima, carboxylic acids, phenols, aromatic amines, alkyl, aryl, ether, secondary alcohols, anhydrides, 1,3-substituted/1,2, 4-trisubstitutions and 1,4 substitution/1,2,3,4 tetrasubstitutions. AntiSMASH analysis results showed that *Streptomyces* sp. CRB46 produces as many as many as Six compounds have 100% structural similarity with compounds that have been found in *Streptomyces*, namely: isorenieratene, ectoine, melanin, albaflavenone, geosmin and 7-prenilistatine. Based on these descriptions, it is concluded that *Streptomyces* sp. CRB46 is a novel species of *Streptomyces*. Therefore, the name of *Streptomyces cemorsewuensis* sp. Nov. is proposed for the name of *Streptomyces* sp. CRB46.

Keywords: *Streptomyces*, Rhizosphere, Highland, Bioactive Compound, Whole Genom Sequencing